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TRIPLE COMBINATION OF A PYRIMIDINE CONTAINING NNRTI WITH A NUCLEOSIDE RT
INHIBITOR AND A NUCLEOTIDE RT INHIBITOR

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TRIPLE COMBINATION OF A PYRIMIDINE CONTAINING NNRTI WITH A
NUCLEOSIDE RT INHIBITOR AND A NUCLEOTIDE RT INHIBITOR

5 The present invention concerns the triple combination of a pyrimidine containing
NNRTI with a nucleoside reverse transcriptase inhibitor and a nucleotide reverse
transcriptase inhibitor useful for the treatment of HIV infected patients or for the
prevention of HIV transmission or infection.

10 BACKGROUND OF THE INVENTION

Despite the fact that significant progress has been made by the introduction of HAART
therapy (Highly Active Anti-Retroviral Therapy), resistance of the HIV virus against
nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse
transcriptase inhibitors (NNRTIs), nucleotide reverse transcriptase inhibitors (NtRTIs),
15 protease inhibitors and even the more recent fusion inhibitors is still a major cause of
therapy failure. For instance, half of the patients receiving anti-HIV combination
therapy do not respond fully to the treatment, mainly because of resistance of the virus
to one or more drugs used. Moreover, it has been shown that resistant virus is carried
over to newly infected individuals, resulting in severely limited therapy options for
20 these drug-naïve patients. On the International AIDS Conference in Paris in July 2003
researchers released that the biggest study so far of resistance to AIDS drugs finds that
about 10 percent of all newly infected people in Europe have drug-resistant strains.
Smaller tests to determine the spread of resistance have been done in the high-risk city
center of San Francisco. This test showed the highest level of resistance at 27 percent.

25 The pharmacokinetic profile of many commercially available antiretrovirals does not
allow relatively low therapeutic doses. Poor pharmacokinetic profiles often in
combination with poor solubility properties of the antiretrovirals cause the AIDS
patient to face a high pill burden which is particularly undesirable for drug-naïve
patients or first line therapy. Moreover, as a consequence of the AIDS virus resisting
30 even antiretroviral combination therapy, a physician will boost the plasma levels of the
active drugs in order for said antiretrovirals to regain effectivity against the mutated
HIV viruses. The consequence of which is an even higher increase in pill burden.
Boosting plasma levels may also lead to an increased risk of non-compliance with the
35 prescribed therapy and to increased side-effects.

Several attempts have been made to date to design combination regimens. For instance,
the combination of lamivudine (a nucleoside RT inhibitor also named 3TC) at a 150 mg

dose and zidovudine (a nucleotide RT inhibitor also named AZT) at a 300 mg dose, formulated in an oral tablet and dosed twice daily, or the combination of abacavir sulphate at a dose equivalent to 300 mg abacavir (a nucleoside RT inhibitor), lamivudine at a 150 mg dose and zidovudine at a 300 mg dose, formulated in an oral tablet and dosed twice daily.

WO 93/23021 describes therapeutic combinations for the treatment of HIV-infections comprising zidovudine and an agent serving to enhance the antiviral activity against HIV populations otherwise resistant to zidovudine.

WO 96/01110 describes a triple combination of zidovudine, lamivudine and loviride. The latter being a non-nucleoside RT inhibitor of the α -APA class.

WO 03/016306 specifically discloses more than 250 pyrimidine derivative having HIV replication inhibiting properties that act as non-nucleoside RT inhibitors (NNRTIs) having the ability to inhibit the replication both wild-type and of mutant strains. One of said NNRTIs is the 4-[[4-[[4-(2-cyanoethenyl)-2,6-dimethylphenyl]-amino]-2-pyrimidinyl]-amino]-benzonitrile (herein named compound A). WO 03/016306 also discloses the methods to synthesize these compounds. It further discloses combinations of said NNRTIs with other antiretrovirals, i.e. suramine, pentamidine, thymopentin, castanospermine, dextran (dextran sulfate), foscarnet-sodium (trisodium phosphonoformate), zidovudine (3'-azido-3'-deoxythymidine, AZT), didanosine (2',3'-dideoxyinosine; ddI), zalcitabine (dideoxycytidine, ddC), lamivudine (2',3'-dideoxy-3'-thiacytidine, 3TC), stavudine (2',3'-didehydro-3'-deoxythymidine, d4T), abacavir, nevirapine (11-cyclopropyl-5,11-dihydro-4-methyl-6H-dipyrido-[3,2-b : 2',3'-e] [1,4]diazepin-6-one), efavirenz, delavirdine, TMC-120, TMC-125, tenofovir, (S)-8-chloro-4,5,6,7-tetrahydro-5-methyl-6-(3-methyl-2-butenyl)imidazo-[4,5,1-jk][1,4]benzodiazepine-2(1H)-thione, α -[(2-nitrophenyl)amino]-2,6-dichlorobenzene-acetamide, RO-5-3335, indinavir, ritonavir, saquinavir, lopinavir (ABT-378), nelfinavir, amprenavir, TMC-126, BMS-232632, VX-175, T-20, T-1249, AMD-3100 and hydroxyurea.

Notwithstanding existing combination therapy, there is still a need in the art for improved antiretroviral therapy, more particularly AIDS therapy. The need in the art is particularly acute for therapy that is effective not only on wild type HIV virus, but also on the increasingly more common resistant HIV viruses. It is thus highly desirable for especially first line therapy to design a combination regimen with a low pill burden that

limits or even suppresses the recurrence of drug resistant virus and which can be used and remains effective for a long term.

5 It is an object of the invention to provide a combination of more than one therapeutically effective antiretroviral drug which combination can be used as first line therapy in drug-naïve patients for a long period of time.

10 It is also an object of the invention to provide a combination of more than one therapeutically effective antiretroviral drug in which the antiretroviral drugs have a complementary resistance profile thus creating a high resistance barrier and thus allowing a drug-naïve patient to take the combination for a long period of time.

15 Another object of the invention is to provide a combination of more than one therapeutically active antiretroviral drug wherein each of the active antiretroviral drugs of the combination can be administered once daily thus reducing the pill burden for the patient.

20 A further object of the invention is to provide a combination of more than one therapeutically active antiretroviral drug wherein each of the active antiretroviral drugs of the combination can be co-formulated.

25 Yet a further object of the invention is to provide a combination of more than one therapeutically active antiretroviral drug wherein a therapeutically effective amount of each of the active antiretroviral drugs of the combination can be co-formulated in one single pharmaceutical formulation.

30 Another object of the present invention is to provide a combination of more than one active antiretroviral drug which combination can be used to prevent HIV transmission or infection in humans.

All references cited herein are incorporated by reference.

DESCRIPTION OF THE INVENTION

35 It has been discovered that compound (A) is a potent reverse transcriptase inhibitor that has an extremely high genetic barrier in combination with a favourable pharmacokinetic profile allowing once daily dosing. Based on this discovery, a triple combination is provided comprising (i) compound (A) or its stereoisomeric form or pharmaceutically acceptable salt or its prodrug, and (ii) a nucleoside reverse transcriptase inhibitor, and (iii) a nucleotide reverse transcriptase inhibitor,

characterized in that, compound (A) and the nucleotide reverse transcriptase inhibitor and the nucleoside reverse transcriptase inhibitor are therapeutically effective HIV inhibitors at a dose that can be administered once daily.

- 5 It was surprising to discover that compound (A) has all these properties together. This is unusual because one cannot predict what mutations will be selected in the HIV-1 genome by a given drug, whether the mutated virus will have any chance of survival under the pressure of the drug, how much drug is needed to limit or to suppress the recurrence of such mutated virus, and at what frequency such drug has to be given to
10 maintain suppression of the development of a resistant virus that can break through the genetic barrier of the drug.

- Advantageously, the nucleotide reverse transcriptase inhibitor and the nucleoside reverse transcriptase inhibitor select mutations in the reverse transcriptase that do not
15 cause resistance to compound (A). Therefore, in a preferred embodiment, a triple combination is provided comprising (i) compound (A) or its stereoisomeric form or pharmaceutically acceptable salt or its prodrug, and (ii) a nucleoside reverse transcriptase inhibitor, and (iii) a nucleotide reverse transcriptase inhibitor, characterized in that, (1) compound (A) and the nucleotide reverse transcriptase
20 inhibitor and the nucleoside reverse transcriptase inhibitor are therapeutically effective HIV inhibitors at a dose that can be administered once daily and (2) the nucleotide reverse transcriptase inhibitor and the nucleoside reverse transcriptase inhibitor select mutations in the reverse transcriptase that do not cause resistance to compound (A).

- 25 Compound (A) can also be used in a method for treating HIV infected patients that includes administering compound (A) or its stereoisomeric form or pharmaceutically acceptable salt or its prodrug, in combination with a nucleoside reverse transcriptase inhibitor, and a nucleotide reverse transcriptase inhibitor, in which method a therapeutically effective amount of compound (A), the nucleotide reverse transcriptase
30 inhibitor and the nucleoside reverse transcriptase inhibitor can be administered once daily. One embodiment of the present invention provides for the present combination for use as a medicine. In another embodiment, the combination of the present invention can be used in the manufacture of a medicament to treat HIV infected patients.

- 35 The present triple combination is especially useful for the treatment of AIDS and related clinical conditions such as AIDS related complex (ARC), progressive generalised lymphadenopathy (PGL) or AIDS related neurological conditions such as

multiple sclerosis. The present triple combination may be particularly useful for the treatment of drug-naïve HIV infected patients.

5 The present triple combination is also useful for the prevention of HIV transmission or infection in humans, in particular sexual transmission. Thus, the present invention relates to the use of a triple combination according to the present invention for the manufacture of a medicament for the prevention of HIV infection or transmission via sexual intercourse or related intimate contact between partners. The invention also relates to a method of preventing HIV infection or transmission via sexual intercourse
10 or related intimate contact between partners comprising administering to a subject in need thereof an effective amount of a triple combination according to the present invention.

15 In a preferred embodiment, each of the ingredients of the present triple combination can be co-formulated in one pharmaceutical form and do not have to be administered in a separate pharmaceutical form. The daily therapeutic antiretroviral amount of the ingredients of the present triple combination of such co-formulated single pharmaceutical form may then be given in a single unit dosage form or even in multiple unit dosage forms, such as two, three, four, five or even more unit dosage forms. A
20 physician will be able to determine the exact dosage to be given taking into account the severity of the patient's condition as well as the patient's weight, gender and possibly other parameters such as individual differences in absorption, biodistribution, metabolism and excretion rates for each drug as well as other factors known to those skilled in the art.

25 Thus, in one embodiment, a pharmaceutical composition is provided comprising a pharmaceutically acceptable carrier and as active ingredients (i) compound (A) or its stereoisomeric form or pharmaceutically acceptable salt or its prodrug, and (ii) a nucleoside reverse transcriptase inhibitor, and (iii) a nucleotide reverse transcriptase
30 inhibitor.

Preferred nucleotide reverse transcriptase inhibitors that can be used in the present triple combination and methods employing such triple combination include tenofovir and its prodrug tenofovir disoproxil fumarate.

35 Tenofovir is an adenosine nucleotide analogue currently commercially available with activity against retroviruses. Tenofovir disoproxil fumarate (tenofovir DF) is a once-daily, orally administered prodrug of the intravenously administered antiviral agent tenofovir (PMPA). For antiviral activity, tenofovir DF needs to be hydrolysed to the

ANP analogue and then phosphorylated to the active diphosphate moiety [Arimilli et al. *Antiviral Chemistry and Chemotherapy* 1997, 8 :6 (557-564); Fridland et al. *Antiviral Research* 1997, 34]. After entry in to lymphocytes or macrophages, the prodrug is quantitatively converted to the parent analogue, tenofovir, and phosphorylated to mono- and diphosphate metabolites. The cellular enzymes that are responsible for phosphorylation of this drug are adenylate kinase and nucleoside diphosphate kinase [Robbins et al. *Antimicrobial Agents and Chemotherapy* 1995, 39 :10 (2304-2308); Robbins et al. *Antimicrobial Agents and Chemotherapy* 1998, 42 :3 (612-617)]. Unlike other nucleoside analogues, such as zidovudine or stavudine, both of whose phosphorylation is cell cycle-dependent, tenofovir is efficiently phosphorylated in resting as well as cycling peripheral blood lymphocytes [Robbins et al. 1998]. Tenofovir can inhibit HIV-1 replication in different cell types that may target HIV, including primary human blood lymphocytes and macrophages [Perno et al. *Antiviral Research* 1992 (289-304); Perno et al. *Molecular Pharmacology* 1996, 50 :2 (359-366)]. The primary target of tenofovir diphosphate is reverse transcriptase (RT). Tenofovir diphosphate is a competitive inhibitor for the incorporation of deoxyadenosine triphosphate into nascent proviral DNA chains. Inhibition of HIV-1 RT by tenofovir diphosphate has an inhibition constant of approximately 0.9 μM , and if the analogue is incorporated into the growing viral DNA chain it may terminate further chain elongation. Tenofovir inhibits viral RT much more effectively than it inhibits cellular DNA polymerases [Suo et al. *Journal of Biological Chemistry* 1998, 273 :42 (2750-2758)]. The concentration required to inhibit the replication of various HIV-1 strains by 50% (EC₅₀) in lymphocyte and macrophage cell types (MT-2, CEM, ACH8) ranges from 0.2 to 10 μM . The antiviral effect is achieved at non-toxic doses of tenofovir (selectivity index ranging from 100 to 2000). Tenofovir DF is currently available as 300 mg tablets to be taken once daily.

Viral resistance to tenofovir in vitro emerges slowly. A recombinant virus expressing the K65R mutation showed a 3-fold decreased susceptibility to tenofovir in vitro [Cherrington et al. *Interscience Conference on Antimicrobial Agents and Chemotherapy* 1997, 37th]. Notably, clinical HIV strains expressing the M184V lamivudine-associated resistance mutation on RT show wild-type or increased susceptibility to tenofovir in vitro, independent of changes in K_i for the mutant enzyme [Miller et al. *Interscience Conference on Antimicrobial Agents and Chemotherapy* 1998,]. Long-term treatment (5 to 15 weeks) of newborn rhesus macaques with tenofovir (doses of 30 mg/kg) starting 3 weeks after inoculation with simian immunodeficiency virus, resulted in emergence of SIV with approximately 5-fold decreased susceptibility to tenofovir [van Rompay et al. *Antimicrobial Agents and*

Chemotherapy 1996, 40 :11 (2586-2591)]. This low level of resistance was associated with the appearance of the K65R mutation.

5 In a preferred embodiment, a triple combination is provided comprising (i) compound (A) or its stereoisomeric form or pharmaceutically acceptable salt or its prodrug, and (ii) a nucleoside reverse transcriptase inhibitor, and (iii) tenofovir or its prodrug tenofovir disoproxil fumarate, characterized in that, compound (A) and the nucleoside reverse transcriptase inhibitor and tenofovir or its prodrug tenofovir disoproxil fumarate are therapeutically effective HIV inhibitors at a dose that can be administered
10 once daily.

In a preferred embodiment, a triple combination is provided comprising (i) compound (A) or its stereoisomeric form or pharmaceutically acceptable salt or its prodrug, and (ii) a nucleoside reverse transcriptase inhibitor, and (iii) tenofovir disoproxil fumarate,
15 characterized in that, compound (A) and the nucleoside reverse transcriptase inhibitor and tenofovir disoproxil fumarate are therapeutically effective HIV inhibitors at a dose that can be administered once daily.

Preferred nucleoside reverse transcriptase inhibitors that can be used in the present triple combination and methods employing such triple combination include
20 emtricitabine, racemic FTC and lamivudine (also named 3TC).

Emtricitabine or (-)-FTC is the left (-) rotatory enantiomeric form of racemic FTC or (\pm)-cis-4-amino-5-fluoro-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-2(1H)-
25 pyrimidinone (FTC). It is a commercially available nucleoside analogue and exhibits activity against HIV-1 [Hoong et al. *Journal of Organic Chemistry* 1992 (5563-5565); Jeong et al *Journal of Medicinal Chemistry* 1993, 36 :2 (181-195); Van Roey et al. *Antiviral Chemistry and Chemotherapy* 1993, 4 :6 (369-375)]. The in vitro anti-HIV-1 activity of (-)-beta-enantiomer of FTC was reported to be 20-fold more than the (+)-
30 beta-enantiomer, and the (+)-enantiomer was significantly more toxic than the (-)-enantiomer to myeloid progenitor cells [Schinazi et al *Antimicrobial Agents and Chemotherapy* 1992, 36 :11 (2423-2431)]. The potential for HIV-1 resistance to FTC was evaluated by serial passage of the virus in human PBMCs and MT-2 cells in the presence of increasing drug concentrations. Highly drug-resistant HIV-1 variants
35 dominated the replicating virus population after two or more cycles of infection. RT derived from drug-resistant viral particles was 15- to 50-fold less susceptible to the 5'-triphosphate of FTC compared with the enzyme from parental drug susceptible virus. DNA sequence analysis of the RT gene amplified from resistant viruses

consistently identified mutations at codon 184 from Met (ATG) to Val (GTG or GTA) [Schinazi et al *Antimicrobial Agents and Chemotherapy* 1993, 37 :4 (875-881); Tisdale et al *Antiviral Research* 1993, 20 :Suppl 1; Smith et al *Journal of Virology* 1997, 71 :3 (2357-2362); Harrer et al *Journal of Infectious Diseases* 1996, 173 :2 (476-479); Tisdale et al *Proceedings of the National Academy of Sciences of the United States of America* 1993, 90 :12 (5653-5656)]. Due to this observed single mutation in the YMDD of reverse transcriptase in the HIV-infected patients, (-)-FTC is not suitable for monotherapy and needs to be administered in combination with other antiretroviral agents to effectively treat patients infected with HIV. Emtricitabine is available as 200 mg capsules to be taken once a day.

Lamivudine has the chemical name (-)-2',3'-dideoxy-3'-thiacytidine and is described for instance in EP 382,526 as an antiviral nucleoside analogue. It is also a well established and useful antiretroviral which is commercially available for instance as 150 mg oral tablets. Lamivudine is also commercially available in combination with zidovudine (300 mg zidovudine / 150 mg lamivudine), and in combination with lamivudine and abacavir sulphate (300 mg zidovudine / 150 mg lamivudine / equivalent of 300 mg abacavir).

In a preferred embodiment, a triple combination is provided comprising (i) compound (A) or its stereoisomeric form or pharmaceutically acceptable salt or its prodrug, and (ii) emtricitabine, and (iii) a nucleotide reverse transcriptase inhibitor, characterized in that, compound (A) and the nucleotide reverse transcriptase inhibitor and emtricitabine are therapeutically effective HIV inhibitors at a dose that can be administered once daily.

In another preferred embodiment, a triple combination is provided comprising (i) compound (A) or its stereoisomeric form or pharmaceutically acceptable salt or its prodrug, and (ii) lamivudine, and (iii) a nucleotide reverse transcriptase inhibitor, characterized in that, compound (A) and the nucleotide reverse transcriptase inhibitor and lamivudine are therapeutically effective HIV inhibitors at a dose that can be administered once daily.

In another preferred embodiment, a triple combination is provided comprising (i) compound (A) or its stereoisomeric form or pharmaceutically acceptable salt or its prodrug, and (ii) emtricitabine, and (iii) tenofovir or its prodrug tenofovir disoproxil fumarate, characterized in that, compound (A) and emtricitabine and tenofovir or its

prodrug tenofovir disoproxil fumarate are therapeutically effective HIV inhibitors at a dose that can be administered once daily.

The following preferred triple combinations are also included

- 5 (a) compound (A) or its stereoisomeric form or pharmaceutically acceptable salt or its prodrug with emtricitabine and tenofovir disoproxil fumarate;
 - (b) compound (A) or its stereoisomeric form or pharmaceutically acceptable salt or its prodrug with lamivudine and tenofovir disoproxil fumarate.
- 10 A preferred form of compound (A) is the E-isomer, i.e. (E)- 4-[[4-[[4-(2-cyanoethenyl)-2,6-dimethylphenyl]-amino]-2-pyrimidinyl]-amino]-benzonitrile (hereinafter called compound E-(A)). The Z-isomer of compound (A) (hereinafter called compound Z-(A)) has relatively high potency against wild-type HIV-1, and also finds use in the present triple combinations. However, it is less active against single and double
- 15 mutants in comparison to the E-isomer. Table 1 shows the IC₅₀ value in nM of the E and Z-isomer of compound A.

Table 1

HIV RT mutation	E-isomer	Z-isomer
Wild-type	0.4	0.6
100I	0.4	6.3
103N	0.3	1.6
181C	1.3	5.0
188L	2.0	32
227C	2.0	4.0
100I+103N	7.9	790
103N+181C	1.0	40
227L+106A	1.0	4.0

- 20 The respective daily dose for each of the compounds of the present combination may range between 10 mg and 800 mg, preferably between 50 and 400 mg, more preferably between 100 and 300 mg.

- 25 The weight ratio of each couple of components of the triple combination taken on a daily basis may vary in a range from 1/10 to 10/1. Suitably, the weight ratio of each couple varies between 1/4 and 4/1, preferably between 1/3 and 3/1, and more preferably between 1/2 and 2/1.

The compounds of the present triple combination may be administered simultaneously, concurrently or sequentially. Simultaneous administration may be done by employing a unitary pharmaceutical formulation or separate pharmaceutical formulations. In general, the combination may be administered by topical, oral, rectal, intravenous, subcutaneous or intramuscular routes. For first line therapy of HIV infection, simultaneous administration employing a unitary pharmaceutical formulation is preferred.

The present invention also relates to a pharmaceutical composition in a form adapted to be applied to a site where sexual intercourse or related intimate contact can take place, such as the genitals, rectum, mouth, hands, lower abdomen, upper thighs, especially the vagina and mouth, comprising a pharmaceutically acceptable carrier and as active ingredients an effective amount of a triple combination according to the present invention. As appropriate special adapted compositions there may be cited all compositions usually employed for being applied to the vagina, rectum, mouth and skin such as for example gels, jellies, creams, ointments, films, sponges, foams, intravaginal rings, cervical caps, suppositories for rectal or vaginal application, vaginal or rectal or buccal tablets, mouthwashes. To prepare such pharmaceutical compositions, an effective amount of each of the particular compounds of the triple combination as the active ingredients is combined in intimate admixture with a pharmaceutically acceptable carrier, which carrier may take a wide variety of forms depending on the form of administration. In order to increase the residence time of such pharmaceutical composition at the site of administration, it may be advantageous to include in the composition a bioadhesive, in particular a bioadhesive polymer. A bioadhesive may be defined as a material that adheres to a live biological surface such as for example a mucus membrane or skin tissue.

Thus, the present invention also relates to a pharmaceutical composition comprising a pharmaceutically acceptable carrier and as active ingredients an effective amount of each of the compounds of the present triple combination characterized in that the pharmaceutical composition is bioadhesive to the site of application. Preferably, the site of application is the vagina, rectum, mouth or skin, most preferred is the vagina.

In order to demonstrate the ability of compound A to prevent HIV infection via sexual intercourse or related intimate contact between partners, compound A can be tested in the following test. Immature monocyte derived dendritic cells (immMO-DC) represent a good model for interstitial dendritic cells, which are early targets during sexual HIV transmission and important initiators of the immune response. These immMO-DC

were used in "in vitro" models to test the prevention of HIV infection via sexual intercourse or related intimate contact between partners. One such model is described in the experimental part and indicates that the compound A potently inhibits HIV replication in MO-DC/ CD4(+) T cell co-cultures.

5

EXPERIMENTAL PART

Pharmacokinetics of compound E-(A)

A double-blind, randomized, placebo-controlled Phase I trial was designed to evaluate safety, tolerability, and *ex-vivo* pharmacokinetics of single doses of compound E-(A) in healthy male volunteers. Oral doses of 12.5, 25, and 50 mg were formulated in PEG 400 and taken with a standard meal. The pharmacokinetic results are shown in Table 2.

The pharmacokinetic results of another double-blind, randomized, placebo-controlled Phase I study with 4 dosing sessions to evaluate the safety, tolerability, pharmacokinetics and *ex-vivo* pharmacodynamics of single 100 mg and 200 mg oral doses of compound E-(A) in healthy male subjects are also reported in Table 2. Randomization was such that for each session 6 subjects received the same dose of compound E-(A) and 3 subjects received placebo. There was a time interval of about 14 days between each dosing session

Table 2 shows that high and dose-proportional exposures were obtained. The correlation coefficient for the 5 C_{max} datapoints is 0.9897 and for the area under the curve values between 0 and 48 hours (AUC_{0-48hr}) 0.9952. Half-life of plasma concentrations ranged between 37 and 39 hours. The compound was well tolerated by the volunteers. No relevant adverse effects of the drug were noted.

10

Table 2

Parameter	12.5 mg	25 mg	50 mg	100 mg	200 mg
C_{max} (ng/ml)	73±14	149±32	267±27	482±121	807 ±207
T_{max} (hr)	4.0±0	4.0±1.3	4.0±1.3	4.3±0.8	4.3 ±0.8
AUC_{0-48hr} (nghr/ml)	1337±310	2805±496	5094±509	8162±2251	15592±2746
$AUC_{0-\infty}$ (nghr/ml)	2210±473	4637±1164	8872±1342	15844±4592	
$T_{1/2}$ (hr)	37.1	38.7	45±9	55±18	

Virological profile of Compound E-(A)

Compound E-(A) was tested in a cell-based assay, using natural host cells of HIV. MT-4 cells (a cell line of human T cells) were infected with HIV-1 (wild type or mutants)

and exposed to different concentrations of antiviral compound in the presence of 10% fetal calf serum. Cytotoxicity was determined in parallel with the antiviral activity so that the selectivity of the antiviral effect could be assessed. Active compounds have to penetrate the cell membrane in order to interfere with replication steps inside the cell.

- 5 After four days of incubation at 37°C, the viability of the HIV and mock-infected cells was assessed by an automated tetrazolium-based colorimetric assay. This method enabled the calculation of both the 50% inhibitory concentration for inhibition of viral cytopathicity (IC₅₀), the IC₉₀, and the 50% cytotoxic concentration (CC₅₀). The ratio CC₅₀/IC₅₀, also called the selectivity index, is an indication of the specificity of the
10 antiviral effect. Tested HIV strains included: Wild type (wt) HIV-1; a panel of single and double mutants, obtained by site-directed mutagenesis (SDM), and a panel of clinical isolates, selected for resistance against NNRTIs.

Activity towards wild type and SDM mutants

- 15 A limited panel of HIV-1 mutants was constructed using site-directed mutagenesis (SDM) and homologous recombination techniques. Compound E-(A) was tested against an extended panel of single and double mutants known to be resistant against commercially available NNRTIs. Nevirapine (NVP) and efavirenz (EFV) were
20 included as controls.

- 20 The results are shown in Table 3 (values presented are IC₅₀ values in nM). For wild type virus, the obtained IC₅₀ was 0.4 nM (0.15 ng/ml) and the IC₉₀ 1.3 nM (0.48 ng/ml). The HIV strain with the lowest sensitivity against compound E-(A) within this selection was the double mutant 100I+103N, with an IC₅₀ of about 8 nM and an IC₉₀
25 of about 16 nM.

Table 3

	NVP	EFV	Compound E-(A)
wild type	81	1.4	0.4
100I	597	35	0.4
101E	547	5	1.6
103N	2,879	28	0.3
106A	2,983	23	0.2
108I	-	2	0.3
138K	64	1.3	0.4
179D	161	6	0.6
179E	158	5	0.4
181C	10,000	2	1.3
188C	3,764	5	0.1
188H	241	9	0.2
188L	10,000	78	2.0
190A	4,101	8	0.3
190S	10,000	275	0.1
225H	171	2	0.3
227C	1,816	36	2.0
227L	78	0.3	0.3
234I	45	NT	0.3
236L	41	1	0.3
100I+103N	10,000	10,000	7.9
101E+103N	7,033	84	0.5
103N+181I	10,000	37	1.0
227L+106A	10,000	8	1.0

Development of resistance in vitro

5 NNRTIs are highly selective inhibitors of HIV-1 but their current clinical use is limited by the rapid emergence of NNRTI (cross-) resistance. The rate of resistance emergence against compound E-(A) and the first generation NNRTIs nevirapine and efavirenz was compared in vitro.

10 MT4 cells were infected with wild type HIV-1 at high multiplicity of infection (>1 infectious virus per cell, to maximize the genetic diversity of the virus population) in the presence of various concentrations of compound E-(A) (40, 200, 1000 and 5000 x IC₅₀), and were monitored twice a week for virus replication. Emerging virus was collected for pheno- and genotyping. Cultures without evidence of virus replication were further sub-cultivated in the presence of the same concentration of inhibitor for a total duration of 30 days (10 passages).

Resistance to nevirapine emerged within 3-6 days, at all tested concentrations.

Breakthrough virus harboured the typical Y181C mutation. The same experiments with efavirenz resulted in the selection of G190E at all concentrations (up to 5 μ M) within 3 to 7 days. Compound E-(A) did not select for resistant virus within 30 days using wild-type virus. If a double resistant mutant K103N+Y181C (IC₅₀ 0.8 nM) was used instead of wild type virus, resistance did emerge at all tested concentrations. Starting from the single mutants Y181C (IC₅₀ 1.3nM) or 103N (IC₅₀ 0.3nM), virus breakthrough did not occur at 40 and 200 nM, but did occur at 10 nM.

- 10 In this experimental setting of high genetic diversity, HIV-1, resistant to first generation NNRTIs, was selected very rapidly. Resistant viruses harboured only one mutation. In contrast, emergence of HIV-1, resistant to compound E-(A) was delayed or did not occur.

15 Cardiovascular and pulmonary safety of compound E-(A)

Compound E-(A) had little or no effect on cardiovascular and pulmonary parameters in vivo at plasma levels covering and exceeding the targeted plasma levels in man and at concentrations in vitro covering or exceeding the anti-viral concentration in vitro.

20 In vitro models to test the ability of compound E-(A) to prevent HIV infection via sexual intercourse or related intimate contact between partners.

For instance, in one model, monocyte-derived dendritic cells (MO-DC) were infected for 2 hours with the monotropic HIV strain Ba-L at a multiplicity of infection (MOI) of 10⁻³. After infection, cells were washed 6 times and resuspended in 10% BCS at 400.000 cells/ml. Autologous CD4(+) T cells were purified out of the lymphocyte fraction of the same elutration as the MO-DC and used at a concentration of 2X 10⁶ cells/ml ((ratio MO-DC/CD4(+) T : 1/5).

- 25 cells/ml. Autologous CD4(+) T cells were purified out of the lymphocyte fraction of the same elutration as the MO-DC and used at a concentration of 2X 10⁶ cells/ml ((ratio MO-DC/CD4(+) T : 1/5).
- 30 A serial dilution of a compound of formula (I) (test compound) was added to the MO-DC/ CD4(+) T cell co-cultures. Each experiment was done in 96-well plates, in which each cup contained 50 μ l of MO-DC, 50 μ l of CD4(+) T cells and 100 μ l of test compound. Half of the culture medium, with test compound, was refreshed twice weekly.

Supernatants were analysed in ELISA after 14 days of culture. To determine antiviral activity, the test compound concentration able to suppress 50% of the viral replication at the end of the primary cultures (EC₅₀) was measured. For compound E-(A), the EC₅₀ value was 0.55 nM.

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CLAIMS

1. A combination comprising
 - (i) 4-[[4-[[4-(2-cyanoethenyl)-2,6-dimethylphenyl]-amino]-2-pyrimidinyl]-amino]-benzonitrile, also named compound (A), or a stereoisomeric form or a pharmaceutically acceptable salt or a prodrug thereof, and
 - (ii) a nucleoside reverse transcriptase inhibitor, and
 - (iii) a nucleotide reverse transcriptase inhibitor, characterized in that, compound (A) and the nucleotide reverse transcriptase inhibitor and the nucleoside reverse transcriptase inhibitor are therapeutically effective HIV inhibitors at a dose that can be administered once daily.
2. A combination according to claim 1 wherein compound (A) occurs in its E-isomeric form.
3. A combination according to claims 1 or 2 wherein the nucleotide reverse transcriptase inhibitor and the nucleoside reverse transcriptase inhibitor select mutations in the reverse transcriptase that do not cause resistance to compound (A).
4. A combination according to any one of claims 1 to 3 wherein the nucleotide reverse transcriptase inhibitor is tenofovir or its prodrug tenofovir disoproxil fumarate.
5. A combination according to any one of claims 1 to 4 wherein the nucleoside reverse transcriptase inhibitor is emtricitabine, racemic FTC or lamivudine (also named 3TC).
6. A combination according to any one of claims 1 to 5 wherein the nucleoside reverse transcriptase inhibitor is emtricitabine.
7. A combination according to any one of claims 1 to 6 wherein the combination comprises
 - (i) E-4-[[4-[[4-(2-cyanoethenyl)-2,6-dimethylphenyl]-amino]-2-pyrimidinyl]-amino]-benzonitrile or a pharmaceutically acceptable salt, and
 - (ii) tenofovir or its prodrug tenofovir disoproxil fumarate, and
 - (iii) emtricitabine.
8. A combination according to any one of claims 1 to 5 wherein the nucleoside reverse transcriptase inhibitor is lamivudine.
9. A combination according to claim 8 wherein the combination comprises
 - (i) E-4-[[4-[[4-(2-cyanoethenyl)-2,6-dimethylphenyl]-amino]-2-pyrimidinyl]-

amino]-benzonitrile or a pharmaceutically acceptable salt, and
(ii) tenofovir or its prodrug tenofovir disoproxil fumarate, and
(iii) lamivudine.

- 5 10. A combination according to any one of claims 1 to 9 wherein weight ratio of each couple of components of the triple combination taken on a daily basis may vary in a range from 1/4 to 4/1.
- 10 11. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and as active ingredients (i) compound (A) or its stereoisomeric form or pharmaceutically acceptable salt or its prodrug, and (ii) a nucleoside reverse transcriptase inhibitor, and (iii) a nucleotide reverse transcriptase inhibitor.
12. A combination as claimed in any one of claims 1 to 10 for use as a medicine.
13. Use of a combination as claimed in any one of claims 1 to 10 for the manufacture of a medicament for the prevention of HIV infection or transmission via sexual intercourse or related intimate contact between partners.

ABSTRACT

TRIPLE COMBINATION OF A PYRIMIDINE CONTAINING NNRTI WITH A
NUCLEOSIDE RT INHIBITOR AND A NUCLEOTIDE RT INHIBITOR

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10 The present invention concerns the triple combination of a pyrimidine containing
NNRTI with a nucleoside reverse transcriptase inhibitor and a nucleotide reverse
transcriptase inhibitor useful for the treatment of HIV infected patients or for the
prevention of HIV transmission or infection. It further relates to pharmaceutical
formulations containing such triple combination.